INSTRUCTIONS

CarboxyLink™ Coupling Gel (Immobilized Diaminodipropylamine)

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20266

Number	Description
20266	**CarboxyLink Coupling Gel (Immobilized Diaminodipropylamine),** 25mL
	Support: 4% crosslinked beaded agarose, supplied in a 50% slurry containing 0.02% sodium azide
	Activation Level: 16-20µmol amine/mL of gel
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.
	For Research Use Only.

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Introduction

The Thermo Scientific™ CarboxyLink™ Coupling Gel (Immobilized Diaminodipropylamine) is a crosslinked beaded agarose support derivatized to contain diaminodipropylamine (DADPA). The resulting gel contains reactive primary amines at the end of a long spacer arm (Figure 1) to which molecules may be conjugated. CarboxyLink Coupling Gel enables covalent coupling of molecules containing carboxyl (-COOH) groups to a gel support for use in affinity purification. The gel is ideal for immobilization of peptides for use in affinity purification of antibodies or other binding partners.

Although other amine-reactive methods can be used for attachment of molecules to Immobilized DADPA, the carbodiimide EDC is unique among cross-linkers in enabling conjugation through carboxylates. In near-neutral to acidic pH buffer, EDC effectively reacts with carboxylates to form an intermediate ester that is reactive with nucleophiles such as the primary amines on DADPA. The resulting amide bond between molecule and the gel support is stable and practically leak-proof in most affinity purification methods.

CarboxyLink Coupling Gel is also suitable for immobilization of oligonucleotides at 5´-phosphate groups. In the presence of imidazole, EDC effectively reacts with phosphate groups in much the same manner as with carboxylates.

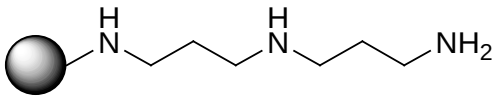

Figure 1. Structure of Thermo Scientific CarboxyLink (Immobilized DADPA) Coupling Gel.

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Important Product Information

- When using EDC, the gel will couple peptides at their C-termini and side chains of aspartic and glutamic acid residues. Because peptides also contain primary amines (the N-terminus and the side chain of lysine residues), coupling using EDC will result in polymerization of peptides as well as their immobilization to the gel support. Usually such polymerization is not detrimental for subsequent affinity purification methods.
- When coupling water-insoluble peptides or other molecules, use water-miscible solvents such as ethanol, methanol, DMSO or DMF. Dissolve the peptide in the water-miscible solvent first, then add this solution to the conjugation buffer. Organic solvent concentrations up to 50% in the coupling reaction are compatible unless the peptide is known to denature in this concentration.
- Traditionally, MES buffer is used for EDC reactions because it does not contain competing amines or phosphates and it effectively maintains optimal acid conditions for coupling. However, other buffers may be substituted if necessary, and coupling will proceed effectively up to pH 7.2. Although phosphate buffer can react with EDC reducing conjugation efficiency, it may be used if compensated by a greater excess of cross-linker. Acetate, Tris and glycine buffers react with EDC or the *O*-acylisourea intermediate and are not appropriate conjugation buffers. Also, avoid using buffers containing thiols, which irreversibly bind and inactivate EDC. Halides such as iodide, chloride and bromide have little effect on EDC between pH 5 and 7.
- The EDC/DADPA method can also be used to immobilize oligonucleotides through their 5´-phosphate groups; for a generalized protocol, see Additional Information section at the end of these instructions.

Procedure for Peptide Immobilization Using EDC and CarboxyLink Coupling Gel

Note: This procedure uses 2mL of CarboxyLink Gel (4mL of slurry) to couple 1-10mg of peptide in a gravity-flow column. For other volumes, peptide amounts and column types, adjust procedure accordingly.

A. Additional Materials Required

- Coupling Buffer: 0.1M MES buffer [2-(*N*-Morpholino)ethansulfonic acid], 0.9% NaCl, pH 4.7 (i.e., Thermo Scientific BupH MES Buffered Saline Pack, Product No. 28390)
- Wash Solution: 1M NaCl
- EDC [1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl] (Product No. 22980)
- Disposable column capable of containing at least 2mL gel bed volume such as the Disposable Polypropylene Columns (Product No. 29922) or the Column Trial Pack (Product No. 29925), which contains two each of three column sizes.

B. Reagent Preparation

Peptide Sample	Dissolve 1-10mg of peptide in 2mL of Coupling Buffer. For estimation of coupling efficiency following peptide coupling, measure the absorbance of this sample at 280nm. (This method assumes that the peptide absorbs at 280nm; other wavelengths that detect the presence of the peptide vs. Coupling Buffer alone may be used).

C. Column Preparation

1. Carefully pack 4mL of CarboxyLink Gel slurry into the column (see instructions for Product No. 29922 for additional information on packing the column). Allow the gel to settle for 30 minutes. Do not insert the top frit.
2. Equilibrate the CarboxyLink Gel columns with 5 column volumes of Coupling Buffer.
3. Replace the bottom cap.

D. Peptide Coupling

1. Equilibrate the EDC vial to room temperature before opening to avoid moisture condensation into the vial.

   Note: Throughout the entire procedure, do not allow the gel bed to become dry; add additional solution or replace the bottom cap whenever the buffer drains down to the top of the gel bed.

2. Add the 2mL of prepared Peptide Sample to the column, place the top cap on the column and mix the Sample/Gel slurry gently end-over-end for several minutes.
3. Add 0.5mL of the Coupling Buffer to 60mg EDC.

   Note: EDC is moisture sensitive and hydrolyzes quickly when dissolved in aqueous buffers. For best results, store the dry powder reagent sealed tightly in its original vial in desiccant at -20°C. Dissolve the required amount of reagent quickly and immediately before use, and discard any unused solution.
4. Immediately after the EDC has dissolved, add the 0.5mL EDC solution to the Sample/Gel slurry from Step 2.
5. Place the top cap on the column and mix the reaction slurry gently end-over-end for 3 hours at room temperature.
6. Stand the column upright. Allow several minutes for the gel to settle, then remove the top and bottom caps and drain the reaction solution from the column into a clean collection tube.
7. Without changing collection tubes, gently add 2mL of Wash Solution to the column and collect the additional 2mL of solution that drains from the column. Replace the bottom column cap.

   Note: The collected sample (~4mL) contains the non-bound peptide. To measure coupling efficiency, compare the absorbance of this solution to the starting Peptide Sample, accounting for the 2-fold dilution effect.
8. With the bottom cap in place, gently add 2mL of Wash Solution to the column so as not to disturb the settled gel bed.
9. Insert a porous polyethylene disc into the column and use the open end of a Serum Separator (or reverse end of a Pasteur pipette) to push it down to 1mm above the settled gel bed.
10. Remove the bottom cap and allow the column to drain into a new collection tube. Column flow will stop automatically when the solution drains down to the top porous disc.
11. If desired, add more Wash Solution (5-10mL) to the column to more thoroughly wash the column of non-bound peptide.
12. Equilibrate the column in an appropriate storage buffer or binding buffer by passing 6mL through the column.
13. For immediate use of the column, equilibrate with a buffer appropriate for the binding interaction. For storage of the column, equilibrate with phosphate-buffered saline (PBS) or other suitable buffer containing 0.05% sodium azide and store the capped column upright at 4°C.

    Note: See Additional Information Section for an example protocol for affinity purification of protein.

Procedure for Nucleic Acid or Oligonucleotide Immobilization Through 5´-Phosphate Groups

Note: The following protocol is presented as a batch method. Use 1µL of gel (2µL of mixed slurry) for each 10µg of oligonucleotide to be coupled.

A. Additional Materials Required

- Pipetters and pipette tips, including one wide-orifice (cut-tip) pipette tip for dispensing gel slurry
- Microcentrifuge tubes and microcentrifuge
- 0.1M imidazole, pH 6
- Ultrapure DNase- and/or RNase-free water

B. Nucleic Acid or Oligonucleotide coupling

1. Mix the CarboxyLink Gel slurry by end-over-end rotation to achieve a uniform gel suspension. Use a wide-orifice pipette tip to transfer an appropriate volume of CarboxyLink Gel to a microcentrifuge tube.
2. Centrifuge the tube for 2 minutes at low speed (e.g., 1000 × *g*), then carefully remove and discard the supernatant.
3. Wash the gel 3-5 times with 2 gel volumes of ultrapure water, centrifuging and removing the supernatant each time.
4. For each microliter of gel used, dissolve up to 10µg of DNA or RNA in 1µL of 0.1M imidazole, pH 6.
5. Add the nucleic acid solution to the gel and mix well.
6. Weigh and dissolve 1mg of EDC in 67µL of 0.1M imidazole, pH 6.

7. For each microliter of gel used, add 2µL of the EDC solution.
8. Mix the gel reaction by shaking or rotating for 3 hours at room temperature.
9. Centrifuge the tube and remove the supernatant, which contains the non-bound nucleic acid.
10. Wash the gel 3-5 times with 2 gel volumes of water or an appropriate wash solution (e.g., Tris-EDTA), centrifuging and discarding the supernatant each time.

Troubleshooting

Problem	Possible Cause	Solution
Peptide not soluble in Coupling Buffer	Peptide was hydrophobic or not soluble at low pH	Use water-miscible solvent (see Important Product Information) or use phosphate-buffered saline, pH 7.2
Poor coupling efficiency	EDC reagent was degraded (hydrolyzed)	Use EDC reagent immediately after dissolving or purchase new EDC
Poor flow rate in packed column	Air bubbles (large and visible or too small to see) formed in gel bed	Use procedure for removing air bubbles; see Tech Tip procedure on our website - prevent air bubble formation by using only degassed solutions (e.g., solutions that have been subjected to vacuum to remove excess dissolved air)

Appendix

A. Please visit the website for additional information relating to this product including the following items:

- Tech Tip #27: Optimize elution conditions for immunoaffinity chromatography
- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns

B. General Protocol for Affinity Purification of Protein

Note: This protocol assumes use of a gravity-flow column with a gel bed volume of 2mL. For columns with other gel bed volumes, adjust all solution (e.g., sample, wash, elution) volumes proportionately.

C. Column Preparation

1. Equilibrate the peptide-coupled column to room temperature.
2. Remove the top cap first to avoid drawing air into the gel bed.
3. Remove the bottom cap and allow excess storage solution to drain from the column.
4. Equilibrate column by washing it with 6mL of the same buffer in which the sample is diluted; e.g., phosphate-buffered saline (PBS, see Related Thermo Scientific Products).

   Note: For best results, degas buffers to avoid introducing air bubbles into the column, which may impede flow.

D. Sample Purification

1. Apply up to 1.5mL of sample to column and allow it to completely enter gel bed.

   Note: For samples >1.5mL, repeat steps B.1-B.5 with 1.5mL aliquots until the entire sample has been used. Alternatively, the gel can be removed from the column and incubated batch-wise with the entire sample volume, then repacked as a column for Steps B6-10.
2. Apply 0.2mL of sample buffer (for example, PBS) and allow it to enter the gel bed. Replace the bottom cap.
3. Apply 0.5mL of sample buffer (for example, PBS) to the column. Replace the top cap.
4. Allow the column to incubate at room temperature for 1 hour.

5. Remove the top cap, then the bottom cap from the column.
6. Wash the column with 12mL of sample buffer (for example, PBS).
7. Elute the bound protein by applying 8mL of an appropriate elution buffer such as IgG Elution Buffer (Product No. 21004 or 21009) or glycine buffer (100mM, pH 2.5-3.0).
8. Collect 1mL (or 0.5mL) fractions. Neutralize low pH by adding 100µL of 1M Tris, pH 7.5 (or 50µL of 1M Tris, pH 9).
9. Monitor elution by measuring the absorbance of fractions at 280 nm.
10. Pool fractions of interest and exchange into an appropriate storage buffer by desalting or dialysis.

E. Regenerating and Storing the Affinity Column

Note: Regenerate the column as soon as possible after chromatography to prevent damage to the immobilized molecule from the low pH elution buffer.

1. Wash column with 16mL of PBS to remove any residual protein and elution buffer.
2. Equilibrate column with 8mL of an appropriate degassed buffer containing 0.05% sodium azide.
3. Replace the bottom cap, then add 2mL of degassed storage buffer to the column, and cap the top.
4. Store upright at 4ºC.

Related Thermo Scientific Products

28390	**BupH™ MES Buffered Saline Packs**, 10 packs
28372	**BupH Phosphate Buffered Saline Packs**, 40 packs
22980	**EDC [1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride)],** 5g
53154	**UltraLink™ EDC/DADPA Immobilization Kit**
29922	**Disposable Polypropylene Columns,** 100 columns

General References (for EDC coupling chemistry)

Gilles, M.A. *et al*. (1990). Stability of water-soluble carbodiimides in aqueous solution. *Anal Biochem* **184:**244.

Grabarek, Z. and Gergely, J. (1990). Zero-length cross-linking procedure with the use of active esters. *Anal Biochem* **185**:244-8.

Williams, A. and Ibrahim, I.A. (1981). A mechanism involving cyclic tautomers for the reaction with nucleophiles of the water-soluble peptide coupling reagent 1-ethyl-3-(dimethyl aminopropyl) carbodiimide (EDC). *J Am Chem Soc* **103**:7090-5.

Product References

Cawley, N.X., *et al*. (2003). Synthesis and characterization of the first potent inhibitor of yapsin 1. *J Biol Chem* **278(8):**5523-30.